

Protocol for Skull Preparation for Chronic Imaging

1. Before the surgery, the animal is anesthetized with isoflurane (3% in oxygen for induction, and 1.5-2% during the surgery to maintain a breathing frequency around 1 Hz). The body temperature of the animal is kept at 37.5 °C with a feed-back controlled blanket (Harvard Apparatus), and the eyes are covered with eye ointment.
2. Immediately after anesthesia, glycopyrrolate (0.01mg/kg body weight), dexamethasone (0.2mg/kg body weight), and ketoprofen (5mg/kg body weight) are administrated intramuscularly. Glycopyrrolate and dexamethasone relieve respiratory distress and the buildup of mucus secretions in lungs during the surgical procedures when the animal is under anesthesia. Ketoprofen is an analgesic for pain reduction.
3. The anesthetized animal is fixed on a stereotaxic. Before any incision on the scalp, the hair must be cleanly removed with scissors and Nair™. The bald scalp is further cleaned and sterilized with alcohol wipes.
4. The scalp is cut open with surgical scissors and removed to expose both parietal plates as well as the bregma and lambda (Figure 1). Sterile saline is applied to the skull surface immediately after the exposure, and it is critical to keep the entire bone surface covered by saline to insulate it from air.
5. Fascia and connective tissue on the skull were gently removed with forceps and sterile wet cotton tips to avoid any internal bleeding inside the brain. The skull should remain covered under sterile saline during the whole process. At this point, the whole skull should be quite transparent, with blood vessels underneath visible with sharp edges.
6. (Time-sensitive Step) Before applying any glue to the skull, the saline covering the skull is wiped dry with cotton tips. Before the bone turns opaque (usually happens within a

couple seconds after exposing to the air), ultra-violet curable glue (Loctite 4305) is applied to the skull surface with a thin wooden tip. If the skull starts to turn opaque before applying the glue, recover it with saline to restore transparency, and then repeat this step. An example of opaque skull can be seen in Figure 1.

7. A sterile and dry round coverslip of 5-mm diameter (#1 thickness, Electron Microscopy Sciences) is placed on the skull, centered at 2.5 mm lateral, and 2 mm caudal from the bregma point. The coverslip is pressed closely against the skull surface by forceps to minimize the amount of glue between the coverslip and the skull. The glue is left to cure by itself for about 5 minutes without any ultra-violet light, during which time the skull transparency tends to increase visually. Afterwards, an ultra-violet light source (385-515nm, Bluephase Style 20i, Ivoclar vivadent) is used to completely cure the glue, with roughly 1s on and 1s off for 3s. The coverslip is necessary to keep the glue layer as thin as possible (down to $\sim 10\ \mu\text{m}$ at the thinnest part on the skull), and to form a flat interface to reduce aberration.
8. The exposed part of the skull surrounding the coverslip is further covered with dental cement. Figure 1 shows an example of successful preparation. For awake imaging, a head-bar for head fixation during imaging is glued to the exposed parts of the skull surrounding the coverslip by metabond glue.
9. For chronic observation, dexamethasone and ketoprofen are also administrated to the animal in two consecutive days following the surgery.

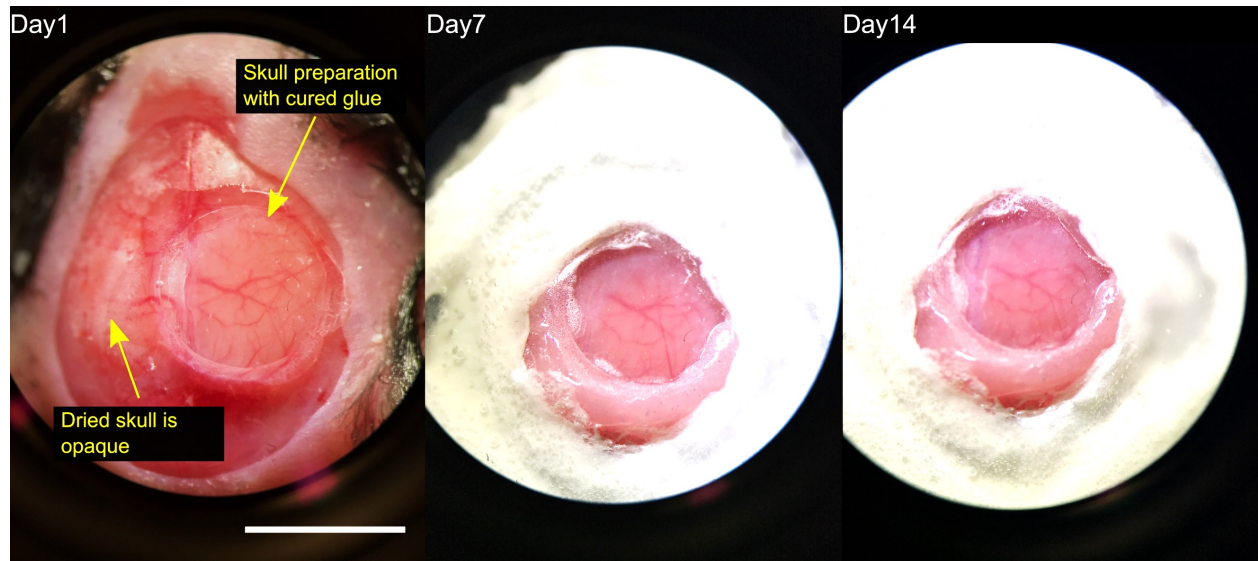


Figure 1. Visual Examination of the Skull Transparency after the Skull Preparation. The bone surface was covered by Loctite 4305, and a #1 round coverslip (5 mm in diameter) was laid on top of it. The glue was cured by UV light. Day 1 was the day of the skull preparation. The images were taken under a stereoscope. Photos on day 7 and day 14 were taken after 2 and 3 recording sessions, respectively. Scale bar, 5 mm.